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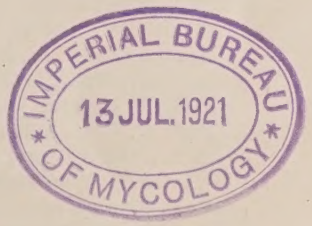
COTTONY ROT OF LEMONS IN CALIFORNIA

BY

CLAYTON O. SMITH



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COTTONY ROT OF LEMONS IN CALIFORNIA*

By CLAYTON O. SMITH

The decay commonly known as "cottony rot" or "white mold," has been associated with the lemon industry probably from its infancy, but during the past few years the trouble has so increased in importance as to demand special study. The disease is widely distributed in all the lemon-growing sections of California, but is not known to occur on Citrus in Florida. The fungus, *Sclerotinia libertiana*, causing this decay, is widely distributed on other hosts and probably is present in other lemon-growing countries. The chief loss occurs during the winter and spring months, when the fruit is being stored and cured in tents before being packed for shipment. No estimate of the loss in money value is obtainable, but where it becomes established in a virulent form, the loss must be considerable. From the records of one of the lemon houses in California the following data of the amount of cottony rot from January to July, 1911, were obtained. During this time 325 cars were in storage. Of these, 97 cars showed amounts varying from 1 to 23 boxes each affected with cottony rot. A box having this trouble means a large percentage of decaying fruit. The decay is more abundant some years than others and especially so when large quantities of fruit are being stored. This is due largely to the greater difficulty in properly inspecting the tents and in detecting the presence of the fungus before it has caused the decay of a large number of fruits by contact with the first infected ones.

The decay on the fruit is chiefly characterized by the mass of white cotton-like mycelial growth that rapidly spreads over the infected lemons. In this growth are to be found black seed-like bodies known as sclerotia, Figs. 1 and 5. By this last characteristic the cottony rot decay can with certainty be distinguished from other lemon rots. In the beginning stages, this decay closely resembles that of the grey mold or Botrytis rot, and at this time the two are often confused in the packing house. In this early stage both of the fungi cause a softening and discoloration of the tissue. The affected tissue, while somewhat softer than normal, is not a typical soft rot, and for some time considerable firmness of the tissue may remain. In the advanced stages,

* Paper No. 22, Citrus Experiment Station, College of Agriculture, University of California, Riverside, California.

however, the tissue becomes more completely broken down and watery.

In the cottony rot as a usual thing the infected tissue is pale lemon in color, while in that of the grey mold it is darker, being almost chocolate. When the aerial growth of the fungi appears, all difficulty in distinguishing between the two decays disappears. In the cottony rot the mycelial growth is white and non-spore producing, but contains hard black sclerotia. In the grey mold (Botrytis) there are numerous spores that can readily be seen in mass. The whole growth and spore masses have a characteristic greyish color. The cottony rot has no distinguishing odor as has the brown rot (*Pythiacystis citrophthora*), so

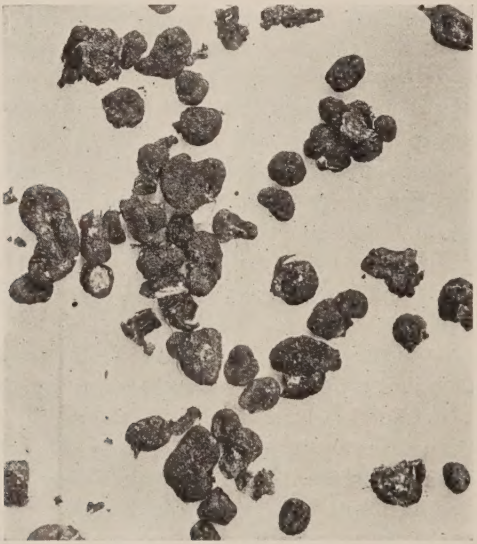

Fig. 1.—Sclerotia of cottony rot, as produced under cover crops. From these the peziza or spore stage grows.

that no difficulty should be had in deciding between the decays of brown and cottony rot.

The twig infection of citrus by the cottony rot is often accompanied with gumming. The infected bark is of a cinerous or ash color and fibrous in its advanced stages. The characteristic sclerotia, Fig. 7*b*, are often, but not always, present. When the twig is at length surrounded by the fungus, death takes place suddenly, the leaves become rolled-up and dead, but may remain attached for some months. This last characteristic is the most noticeable and is what usually attracts the grower's attention to this disease in the orchard.

NATURAL INFECTION

Picking-Box Infection.—It is only occasionally that the conditions are favorable for cottony rot to develop in the picking boxes, as the fruit remains in these only a short time before being washed. Several instances, however, have come to our notice in which, under favorable conditions, a large amount of this decay (possibly as much as 75 per cent) did develop. The fruit was picked and left out in a rain of several days' duration without protection. Under these conditions not only a great amount of decay was to be detected when the fruit was

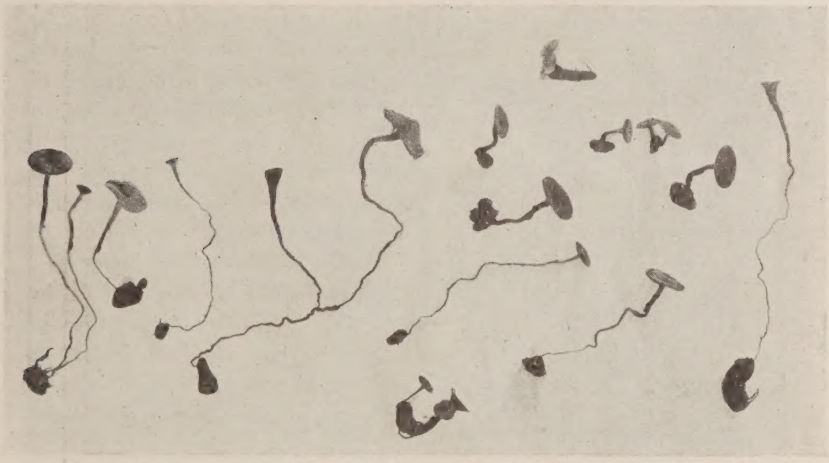

Fig. 2.—Peziza or spore stage showing varying lengths of stem from sclerotia to the funnel-shaped disk, apothecia, of cottony rot fungus. The length of stem varies according to the depth below the surface of the sclerotium.

washed, but this continued to appear during the process of curing. In a few localities the fruit is stored in the picking boxes and no washing of the fruit is considered necessary. In these cases the picking box becomes also the storage box.

Packing-House Infection.—The infection of fruit in the packing house is the chief injury done by the cottony rot fungus. The fruit is left in the curing tents or tight rooms from one to two months after being washed, when the final sorting, grading and packing of the fruit takes place. During this time, especially on fruit unusually weak, the cottony rot has a favorable chance to develop. The decay is found almost entirely during the spring and winter months, January to

May, rarely to June. After this time, the decay almost entirely disappears. The lemons during curing are kept under rather moist conditions to prevent wilting, and in the stacked boxes the conditions are ideal for Sclerotinia to develop. The rot when once started, becomes virulent and a large mass of fruit, even green fruit, may become involved (Figs. 4 and 5). The fungus secretes a substance, perhaps an enzyme, that has the power of softening the tissue of a healthy lemon and so prepares the way for the mycelium to enter. When the disease once becomes started in a half box of lemons, almost every lemon soon becomes infected and the decay often spreads upward and downward, extending through the entire stack of fruit. Workers in the packing house have the idea that the drip from the rotting of the fruit into the box containing sound fruit beneath, has much to do with the spread of the fungus through the stack of fruit. This has never been definitely proved, but without question the mycelium does grow through the open spaces in the bottom of the boxes to sound fruit below.

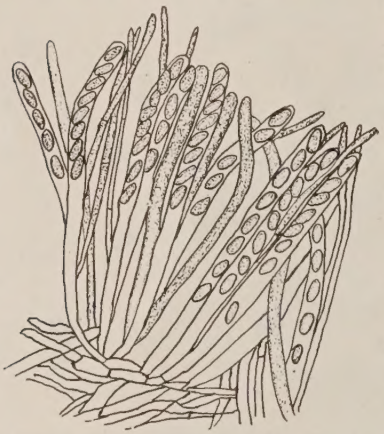

Fig. 3.—Section showing how the spores are produced in long sacks or asci that form the spore-bearing surface of the fungus.

Care should be exercised in the handling of contact fruit; that is, fruit that has been touching cottony rot decay. This will be considered in detail later under "control methods." In most respects the treatment is similar to that recommended for brown rot, and with this most packing house workers are familiar.

Seed-Bed Infection.—The cottony rot is not regarded as a serious disease of citrus seed-bed stock. It has, however, been found occasionally attacking sour and sweet orange seedlings. The shaded moist condition of the seed bed covered with lath or burlap, gives ideal conditions for the fungus to thrive. The disease usually shows in small patches of six to a dozen trees. It is thought to gain entrance into sound tissue by starting as a saprophyte on weakened or fallen leaves that are in close contact with healthy leaves or stems. Trees in the seed bed often grow very close together. Seedlings 1½ to 3 feet high have been found to be attacked, although there is no reason why those smaller or larger might not be infected as easily.

Nursery Infection.—The disease only rarely does any marked injury on citrus nursery trees. It usually attacks trees scattered here and there throughout the nursery and shows no evidence of spreading to adjacent healthy ones (Fig. 6). The diseased trees show the same characteristics as do the infected twigs of orchard trees and should be given the same treatment.

Orchard Infection.—Among citrus trees the lemon is more often attacked than the orange. The disease appears to be localized on a branch, the infection often occurring some distance from the end of the twigs. Smaller twigs are more often infected, although vigorous growing sprouts are also attacked. The twigs may become infected

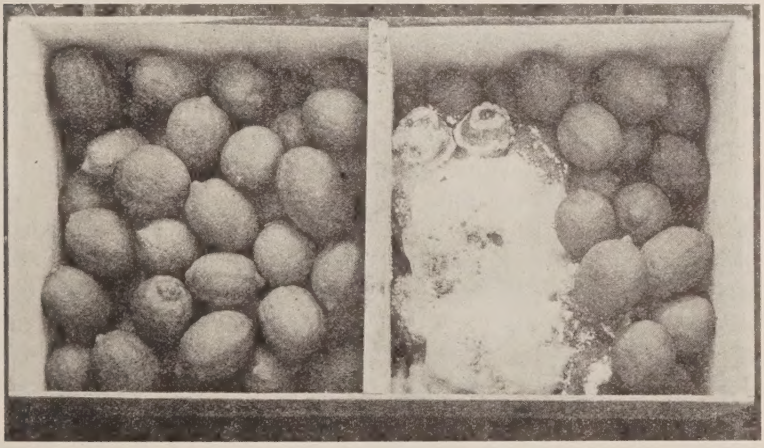

Fig. 4.—Cottony rot affecting lemons by contact in a packing-house box.

through the blossoms or through some injury. The disease is often worse following a cool winter when the tissue is weakened or injured by frost.

Definite infected areas developed on the twig. These increase in size, soon entirely girdling the twig and causing the sudden death of the part beyond the place of infection. The disease does not extend either up or down the infected branch to any great distance. The infected area at first shows a softening of the bark tissue with a considerable amount of gumming (Fig. 7a). The bark of the diseased area is often of a lighter color and at length shows a fibrous condition (Fig. 7c). With the appearance of the gumming of the twig the spread of the infection appears to be checked. On an infected twig the leaves remain for a long time in a rolled-up dead condition (Fig.

8). Small twigs are more frequently attacked, although vigorous growing sprouts may also be infected. The infection may sometimes start at the end of the twig and spread for a short distance back into the more vigorous wood. In these cases the spread of the disease can often be shown to follow injury from frost of the preceding winter, although other conditions, as unusually long periods of warm, rainy weather in winter or spring, also favor twig infection.

The cottony rot fungus has been found occasionally attacking the twigs and trunk of small lemon trees that have been wrapped with corn stalks for winter protection. This fungus has killed young trees that have been planted in the orchard one year from the nursery. A case was observed by Professor H. S. Fawcett in which the fungus had secured a good foot-hold in the trunk and roots of a large lemon tree, which had been weakened by attacks of gummosis. This tree had been wrapped with manure and burlap to keep the tissue from drying out so as better to favor healing, thus affording ideal conditions for fungous growth, and the formation of characteristic sclerotia.

Under favorable conditions the blossoms may become diseased, the infection from here spreading into the fruiting twigs. It is believed that blossom infection most often starts on the older petals which, instead of falling, may remain attached in a more or less decadent condition susceptible to the attack of this fungus (Fig. 9). The blossoms of lemons often grow in large clusters and a considerable mass of collapsed petals are present where spores can germinate and begin growing as a saprophyte, at length acquiring a parasitic nature capable of infecting healthy tissue. This same fungus at times is a parasite on the blossoms of apricots,[1] attacking them while still enclosed by the calyx or outer cup of the blossom.

The leaves of lemons are sometimes attacked while they are attached to the tree. There is not, however, any marked defoliation, probably only the older and weaker leaves being susceptible.

Cover-Crop Infection.—There is no question but what the cottony rot decay has increased with the increased growing of cover crops. The same fungus not only occurs as a rot of the lemon, but is also capable of becoming a parasite on certain cover crops, vetch being by far the most common host. While the fungus is common on vetch, the injury it does to the crop as a whole is negligible. The dense growth of vetch affords a suitable place for the fungus to grow as a saprophyte or weak parasite on the weaker shaded portion of the vines. (Fig. 10) Under the vetch cover crop the sclerotia are produced in large numbers

[1] Bulletin No. 218, California Experiment Station, p. 1097.

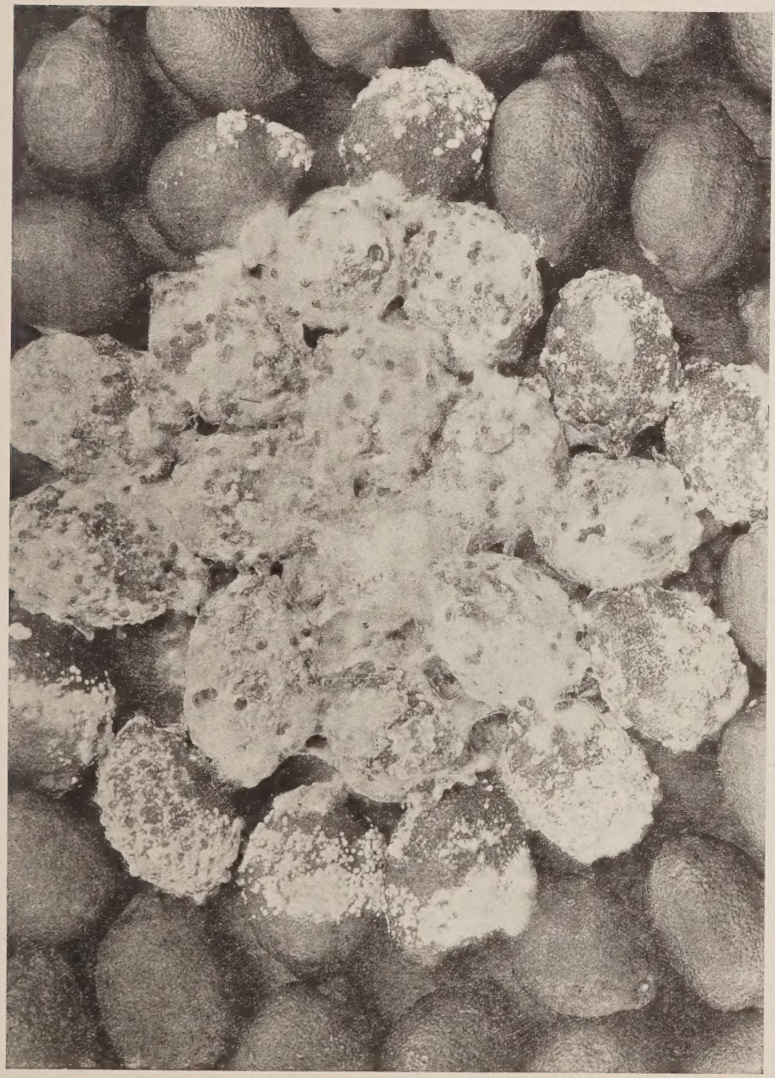

Fig. 5.—Cottony rot affecting lemons by contact in a packing-house tray.

and can be found in infested orchards with little searching. The more upright cover crops, like *Melilotus indica*, would probably be less favorable for the development of the fungus, although this phase of the subject has not been thoroughly investigated. The spore stage has been found in orchards having a straw mulch, but never in so large numbers as with vetch (Fig. 11). There is no question of a definite relationship existing between this fungus and some of the common cover crops in use, but this apparently should not in any way discourage their use. The control of the fungus must be sought for in other ways than through discontinuing the use of green-manure crops.

CAUSE OF DISEASE

The decay, as already suggested, is due to a fungus belonging to the genus Sclerotinia. From the preliminary study thus far made, it is believed that the fungus is *Sclerotinia libertiana*, although another species of this genus, probably *Sclerotinia trifoliorum*, is able to develop on lemons from artificial inoculations and possibly may cause some of the infection.

Relation to other Hosts.—This fungus is not restricted to citrus for a host, but is an important trouble, attacking various other plants, especially seedlings, certain of the vegetables, green-manure plants, and weeds. The following are some of the hosts known to be affected by the lemon Sclerotinia, or one that closely resembles it pathogenically and morphologically: Lemon fruit, twig and blossoms of lemon, orange twigs, apricot blossoms, rarely avocado twigs and blossoms, lettuce, tomato, alfalfa, ginseng, celery, bean, cucumber, vetch, nettle (*Urtica urens*) and prickly lettuce (*Lactuca scariola*), two common weeds in citrus orchards, and occasionally the leaves and weaker stems of barley and oats. As a saprophyte, the fungus could attack, under favorable conditions, many sorts of dead organic matter and decaying plants.

From spore measurements and other microscopic characteristics of the cottony rot fungus, it has always been regarded as the species, *libertiana*. To determine more fully this point, cultures of *Sclerotinia libertiana*, were requested from several of the Experiment Stations. Puncture inoculations from these were made in the twigs of Eureka lemon, as well as on the fruit. The results of these are summarized under artificial inoculations, Table I, p. 252, and in almost every case gave definite, positive results. There remains little doubt of the cottony rot fungus being identical with *Sclerotinia libertiana*.

The following are the different hosts and localities from which these cultures were secured: (1) Several different cultures from

lemon fruits, California; (2) six or more different strains from lemon twigs, California; (3) alfalfa, California and Oregon Experiment Stations; (4) cucumber, California and Oregon Experiment Stations; (5) lettuce, California and Experiment Stations of North Carolina, Maine and Wisconsin; (6) eggplant, California; (7) tomato in greenhouse, California; (8) celery, California; (9) bean stem, Maine Experiment Station; (10) snapdragon, Maine Experiment Station; (11) ginseng, from Cornell University; (12) avocado, California. This last fungus was isolated by Professor H. S. Fawcett from twigs of avocado which had been killed from infection that appeared to have started in the blossoms and spread into the more woody twigs.

Fig. 6.—Eureka lemon tree in nursery affected with cottony rot.

Life History of Fungus.—The life history of the fungus consists of three stages, differing in appearance and functions: (1) The vegetative stage is the white cotton-like growth so common in the packing houses. This growth has no spores, but can spread rapidly and has the power to infect sound fruit simply by coming in contact with and spreading over it. (2) In this white growth, numerous black bodies known as sclerotia are developed (Fig. 1). These are nothing more than a very solid dense mass of mycelium, so formed that they will resist long periods of hot dry weather or the cold freezing conditions of the eastern states. After these bodies have gone through a period of rest and the favorable rainy, cool weather of the autumn and winter

has come, they revive and become active. The sclerotia may then send out a fresh growth of mycelium, the vegetative stage, which could easily infect dead organic matter or the weaker plant growth of weeds

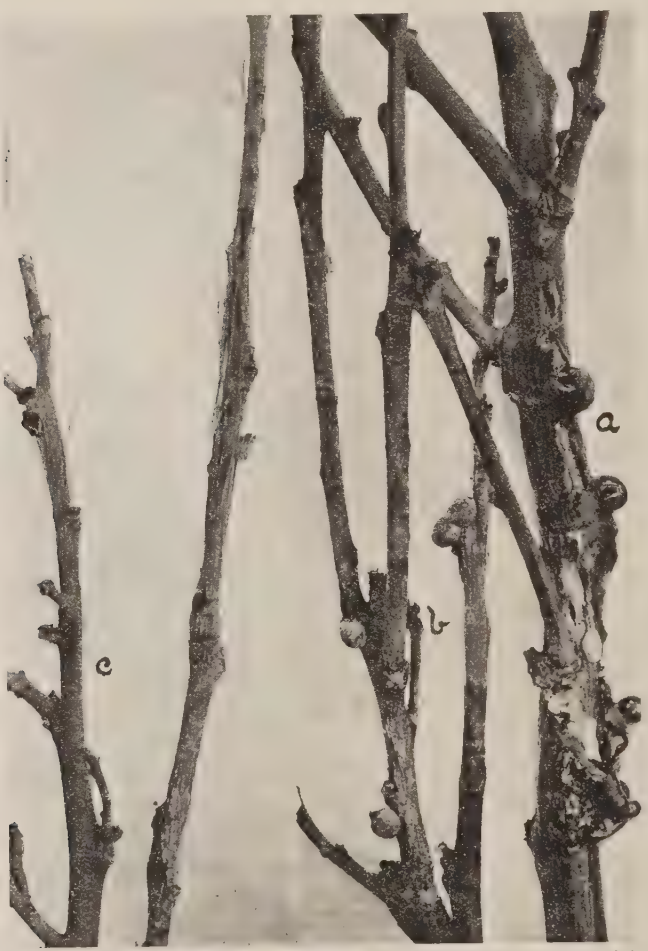

Fig. 7.—Cottony rot affecting the twigs of a Eureka lemon tree. (*a*) Masses of gum produced by the fungus. (*b*) Sclerotia of the fungus distinguished from the gum globules by more spherical shape. (*c*) Fibrous condition of infected bark.

or cover crops. Here after a time new sclerotia are again formed. (3) From the sclerotia in the soil the spore stage is formed under the cover crops in the lemon orchards. This last is accomplished by sending out a growth which eventually develops into a small funnel-

shaped, cup-like disk, technically called an apothecium (Fig. 2). In the flesh of the apothecia are produced the spores. These are formed in rather long cylindrical sacks or asci (Fig. 3). Hence the spores are called ascospores. Both the spores and asci are microscopic. The spores are easily scattered by the wind. The fungus also has the power to force out or discharge these spores, and they can often be observed leaving the apothecium as small clouds of dust. Some of

Fig. 8.—Artificial inoculations of twigs of Navel orange. Note the rolling of leaves which have become dead and bleached to almost a white color and yet still remain attached to branch.

these spores are carried by the wind to the fruit and twigs, of the lemon and cover crops, where under favorable conditions they germinate and produce the vegetative stage.

The apothecial stage in California begins to appear about the first of October and continues to be formed during the winter and spring. After the cover crops of the orchards are plowed under and cultivation begins, few apothecia (spore stage) are thought to be produced, for this stage of the fungus requires moist, shaded conditions, such as are best found under cover crops during the rainy season.

ARTIFICIAL INOCULATION EXPERIMENTS

On Fruit.—The disease can be readily produced on the ripe or green lemons when left in a moist chamber: (1) By placing the mycelium or sclerotia in the tissue through a puncture or injury; (2) by placing mycelium on the uninjured surface of the fruit—this is especially virulent if some other dead organic matter is also present; (3) by placing a piece of an apothecium in the tissue through a puncture or injury; (4) by atomizing spores on the surface, in which case infection occurs only at the button and blossom ends and at points in contact with each other or with the moist chamber; (5) by inoculating the stem and blossom ends with mycelium of the fungus, infection developed in two weeks' time.

Apothecia placed in drops of sterilized distilled water on the surface of lemons gave negative results. Many spores must have been liberated in the water, but the germ tubes were not able to infect the uninjured rind. This observation agrees with those of former investigations that the mycelium of this fungus needs to grow for a time as a saprophyte before it becomes actively parasitic. The reason for this requirement is perhaps explained by the fact that the mycelium secretes an enzyme or substance that apparently plays a very important part in the softening and breaking-down of the tissue, thus preparing the way for its invasion with the fungous hyphae. Spores placed in drops of water or in drops of prune juice give negative results on uninjured tissue.

Typical experiments in inoculating lemons with cottony rot. From these and numerous other experiments conclusions as given under artificial inoculations were largely derived:

1. Experiments in atomizing lemons in a moist chamber with spores of the fungus. This is only one of many made during the investigation. The others, however, show practically the same results.

(*a*) In this experiment an attempt was made to imitate as nearly as possible natural conditions. On February 27th, 1911, five nearly ripe lemons were secured from an orchard that had always been free from this decay. The fruit was picked during a rainy period beginning on February 25th and continuing more or less until March 2d. The fruit was first sterilized for five minutes in 4 per cent formalin* and then rinsed in tap water and left out in the rain for a short time before being atomized with spores. The fruit was then placed in a quart fruit jar for a moist chamber, and left out in the rain where the temperature was somewhat cooler than that in the laboratory. When the rain ceased the jar was covered with a large plant pot to exclude the light. In five days the fruit was examined and found to be infected at

* An aqueous solution of 40 per cent formaldehyde is a commercial product, and is called "Formalin".

all points where it was in contact. Four of the five showed infection at the stem end. At this time there was no evidence of aerial mycelial growth, but simply a small amount of tissue infected and softened at all points where the fruits were touching each other and at the stem end. Characteristic mycelium and sclerotia developed later.

(*b*) Secured from lemon orchard fruit showing the small beginning spot of brown rot (*Pythiacystis citrophthora*). The fruit at this time is only slightly pitted or depressed at point of infection. Sterilized the brown rot fruit in 4 per cent formalin for five minutes and rinsed same in tap water. No injury by germicide was observed on these lemons. On February 25, 1911, this fruit was atomized with spores of cottony rot. On March 8th, the brown rot had spread over about one-third of the surface of the lemons. The cottony rot was also developing over the brown rot areas and had invaded some of the tissue not yet infected by the brown rot fungus. On March 14th, the cottony rot had completely overgrown and covered the entire fruit. The cottony rot seems to attack readily tissue in which the brown rot has become established. In brown rot fruit kept as a check without being inoculated with cottony rot spores, no cottony rot appeared.

2. Experiments in soaking lemons in water inoculated with spores of cottony rot. Experiments were made at several different times following in a somewhat limited way methods similar to those used in inoculating lemons with the brown rot.

A receptacle of sufficient size to contain twelve lemons was filled with sterile water inoculated with macerated apothecia of cottony rot. On December 28, 1910, soaked lemons in this solution for 36 hours, and at the end of two weeks' time there was no evidence of decay. February 3, 1911, the experiment was repeated with thirteen green lemons soaked in tap water mixed with six large apothecia secured from under a vetch cover crop. The apothecia were first macerated in small pieces, and the fruit was left for 24 hours. On March 29, 1911, the fruit showed no evidence of decay.

3. Experiments in inoculating the fruit in moist chamber through punctures and other mechanical injuries. This method rarely fails of giving positive results.

February 15, 1911, inoculated three lemons in moist chamber with pieces of the apothecia, first making a slit in the rind of fruit on side and at stem end. These apothecia came from two different sources: (1) from the orchard under a vetch cover crop; (2) from cultures isolated from a diseased orange branch. February 25, 1911, all the inoculated lemons showed infection at the points of inoculation.

4. Experiment in inoculating fruit by placing mycelium on the uninjured surface.

(*a*) December 28, 1910, inoculated stem or button end of three lemons with mycelium taken from moist chamber where the cottony rot fungus was growing on a lemon. January 15, 1911, slight indication of softening of tissue around stem. February 16, 1911, area about stem had softened in every case and later developed typical cottony rot.

(*b*) December 22, 1910, placed mycelium from a cottony rot lemon on the uninjured surface of five lemons. January 3, 1911, showed diseased, softened areas about an eighth of an inch in diameter in each place where the surface was inoculated. Typical cases of cottony rot soon developed.

TABLE I

SUMMARY SHOWING THE REACTIONS OF HOST TISSUES FROM ARTIFICIAL INFECTIONS WITH AGAR WITH THE MYCELIUM OF VARIOUS STRAINS OF SCLEROTINA FOR THE MOST PART SUPPOSED TO BE SCLEROTINIA LIBERTIANA

Source of culture	Host	Reaction	Twigs	Reaction	Twigs	Reaction	Twigs	Reaction	Twigs	Reaction
Lettuce, California	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Lettuce, North Carolina	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Lettuce, Maine	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Lettuce, Wisconsin	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Ginseng, Cornell	Lemon	Positive	Lemon	Positive	Orange	Positive	Olive	Negative	Plum	Negative
Snapdragon, Maine	Lemon	Positive	Lemon	Positive	Orange	Positive	English walnut	Negative	Plum	Negative
Beans, Maine	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Lemon twigs, California	Lemon	Positive	Lemon	Positive	Apricot	Negative	Peach	Negative	Plum	Negative
Alfalfa, Oregon	Lemon	Positive	Lemon	Positive	Orange	Positive	Avocado	Doubtful	Plum	Negative
Lemon fruit, California	Lemon	Positive	Lemon	Positive	Orange	Positive	Avocado	Doubtful	Plum	Negative
Avocado, California	Lemon	Positive	Lemon	Positive	Orange	Positive	Avocado	Doubtful	Plum	Negative

On Citrus Twigs.—These are very readily infected artificially with the cottony rot fungus. Infection has been repeatedly caused by using apothecia and spores, mycelium and sclerotia. In the inoculation of the twigs a sloping cut through the bark into the wood was made and con siderable of the mycelium was placed in this injury. To insure the best infection, the injury was then wrapped with paraffin waxed paper to prevent drying out. By this method the injured part of the wood and bark would be rather easily attacked by the fungus, starting as a saprophyte. From the injury the infection gradually spreads until

Fig. 9.—Cottony rot affecting the blossoms of a Eureka lemon tree. This condition only appears under certain very favorable conditions.

it completely encircles the branch. Gumming takes place, especially toward the margin of the infection. The affected bark of the citrus twig differs from normal tissue in color, being greyish or cinerous. Sclerotia may or may not be produced in the infected bark (Fig. 7b), which is at first soft, becoming at length fibrous. The infection may extend 1 or 2 inches in length of twig, gradually surrounding and killing it. The infection appears to die out when healing takes place.

The conclusion drawn from these experimental inoculations with different strains of Sclerotinia is: (1) That the different strains used must belong to a common species, or (2) that the citrus host must be especially susceptible to infection from different species of this genus.

METHODS OF CONTROL

In Field.—The spore stage of the fungus occurs in the citrus groves (Fig. 11), never in the packing house. Certain favorable conditions are necessary in the grove for the fungus to continue to perpetuate itself. Vegetable organic matter must be present on which the mycelium can develop during the rainy season. This it does as a saprophyte on the dead parts of plants, gradually making its way into the tissue, killed probably by the secreted enzymes and acids. In this

Fig. 10.—The vegetative stage of cottony rot as it appears growing on a vetch cover crop.

vegetative stage the sclerotia which later produce the spore stage, are formed. Without this organic matter in the orchard in a few years the spore stage would cease to be formed. What has just been said should not in any way be regarded as a recommendation to cease growing cover crops. Most growers would prefer to lose some lemons by cottony rot, even though indirectly brought about through the continual growing of cover crops, rather than to sacrifice the health and vigor of the orchard by not adding vegetable organic matter to the soil by green-manure crops. Observations on fruit grown in clean cultivated groves show little or no cottony rot. The groves where

the decay is worse are those that may have the best care and in which a cover crop or the natural vegetation is allowed to grow. A cover crop gives almost ideal conditions for the sclerotia and the spore stage to develop.

Spraying for this trouble is not to be recommended. All observations thus far show no fruit to be infected on the trees, and the fruit can be more economically disinfected in the wash water at the packing house. Many spores of the fungus must lodge on the surface of fruit and some must germinate while the fruit is attached to the tree, but from the experiments already considered, probably no infection could take place unless it might be at the blossom and button of stem. This is unlikely, as orchard conditions would be much less favorable than those of the packing house or moist chamber. Still it may not be impossible for a slight infection to occur at the places just mentioned. This may account in part for the difficulty of disinfection in the wash water.

The disease on the branches of citrus trees, while of scientific interest, is under ordinary conditions of no great economic importance. Where present the diseased twigs can be readily cut out when the lemon trees are pruned.

In Packing House.—The use of germicides in the wash water as a control for the brown rot, *Pythiacystis citrophthora,* is a general practice. The chemical used most is copper sulphate, which is very efficient as a check for brown rot, but is not equally effective against the spores of the blue and green mold, *Penicillum italicum* and *P. digitatum*, or those of cottony rot, Sclerotinia. The chief reason for this is that the spores are given off in dry masses containing air which forms a film about many of the spores, preventing their being wet by a watery solution of the germicide. This is well illustrated by the following experiment to test the action of copper sulphate as a germicide:

April 5th, 1912. A single apothecium was placed without crushing it in 10 cc. of sterilized water contained in a test tube. After 15 minutes the water was well infected with cottony rot spores, and the apothecium was removed. From this spore-laden liquid, one loopful (2 mm. diameter loop) was placed in each of the test tubes containing 10 cc. of copper sulphate to be tested. After the required length of time that the copper sulphate solution was to act on the spores, the entire contents of the tube was poured into a flask of bread and prune juice medium. The following shows the strengths in per cent used and the time that the spores were acted upon by the germicide:

Per cent	3 min.	5 min.	10 min.	15 min.
1/5	5 tubes	5 tubes	5 tubes	5 tubes
1/10	5 tubes	5 tubes	5 tubes	5 tubes
1/25	5 tubes	5 tubes	5 tubes	5 tubes
1/50	5 tubes	5 tubes	5 tubes	5 tubes

April 11th, 1912. All the different strengths tested in bread-prune juice medium showed characteristic growth of cottony rot. Hence

Fig. 11.—Peziza or spore stage of cottony rot fungus growing under a vetch cover crop in orchard soil.

even the longest time, 15 minutes, and the greatest strength, 1/5 per cent, were not sufficient to kill all the spores treated. It is not strange, therefore, that under the usual commercial conditions, spores of the fungus should occasionally pass through the disinfection, with copper sulphate, without being killed.

The chief difficulty in using disinfectants is to find one that will kill the spores without injuring the lemons. Formaldehyde is much more efficient as a germicide for cottony rot than copper sulphate, but

its use is not to be encouraged because of its injury to the fruit, followed by infection with the two species of Penicillium, the green and blue mold.

In some of the preliminary work it was shown that by using a wash of alcohol preceding that of the copper sulphate, more satisfactory results could be secured in killing the spores of the cottony rot. This method was tested out in a limited way at one of the packing houses. The results are given in another paragraph and suggest that further experimentation may prove that alcohol has some beneficial results in separating the spores from each other so that they may be the more readily acted upon afterwards by the germicide.

The experiment was divided into three parts: (1) Fruit was washed in tap water; (2) fruit was washed in tap water acidified with 255 cc. (about ½ pint) of commercial sulphuric acid to 1000 gallons of water, afterwards copper sulphate was added to make it 1/50 per cent, which is as strong a solution of bluestone as can be used without danger of injuring the fruit; (3) fruit was soaked in 25 per cent denatured alcohol for five minutes and then washed as indicated under "2."

The object of adding the sulphuric acid was to neutralize partly the alkalinity in the wash water. This will be considered in detail later on in this paper. The experiment extends over a portion of two seasons and is best summarized as follows:

Date	Treatment No. 1*	Treatment No. 2†	Treatment No. 3‡	Results 40–60 days
3/7/14	12 boxes	12 boxes	12 boxes	No cottony rot.
3/17/14	12 boxes	12 boxes	12 boxes	1 box cottony rot in Treatment 2.
3/24/14	10 boxes	10 boxes	10 boxes	No cottony rot.
4/4/14	10 boxes	10 boxes	10 boxes	No cottony rot.
4/15/14	10 boxes	10 boxes	10 boxes	No cottony rot.
12/3/14	8 boxes	8 boxes	8 boxes	1 box cottony rot in Treatment 1.
1/7/15	8 boxes	8 boxes	8 boxes	No cottony rot.
1/14/15	8 boxes	8 boxes	8 boxes	No cottony rot.

* Column 1. Contains the number of field boxes washed in plain tap water.

† Column 2. Contains the number of field boxes washed in acidified water, copper sulphate solution 1/50 per cent.

‡ Column 3. Contains the number of field boxes treated first with 25 per cent denatured alcohol, then washed in acidified copper sulphate solution 1/50 per cent.

The work so far is not at all conclusive. No cottony rot developed where alcohol was used, while it did appear in a few cases where the copper sulphate was used in the water or where only water was used.

This may have been accidental. The experiment was inconclusive because so little cottony rot appeared to be present in the fruit.

Germicidal Efficiency of Wash Waters.—Copper sulphate 1/50 per cent is the best germicide thus far tested for general use in the disinfection of lemons, although it is not equally effective in the killing of the spores of the different rot producing fungi, even when kept at the above strength. The fruit goes through the washer in a rather short length of time, so that it is very essential that the strength of the copper sulphate be kept uniformly as strong as can safely be used. The following paragraphs were written on the preparation of disinfecting wash water by Mr. E. E. Thomas, of the Citrus Experiment Station, who has been giving considerable study to the chemistry, and especially to the alkalinity, of the wash waters used in many of the lemon packing-houses in California:

"The main trouble in the use of copper sulphate in the wash water as a disinfectant has been in keeping the strength constant during the day. In the morning it may be one strength, at noon another, and at night still another. This should not be the case, but the percentage of copper sulphate in the water should remain uniformly the same throughout the day in order to give a thorough and satisfactory disinfection of the fruit.

"Most of the wash waters used for washing lemons in the packing-houses of California contain carbonates in solution that give the water an alkaline reaction. These carbonates precipitate a part of the copper sulphate as basic copper carbonate and thus reduce the copper sulphate in solution and lower the germicidal strength of the solution.

"This factor can be largely overcome by the addition of an acid, such as sulphuric acid, to the water before the copper sulphate has been poured into it in the wash tank. The addition of this acid can be accomplished without injury to either fruit or tank, as the desired result can be obtained by neutralizing only two-thirds or three-quarters of the alkali in the water. It would be advisable to add from eight to ten fluid ounces of 91.5 per cent sulphuric acid to a tank containing one thousand gallons of water which has two hundred parts calcium carbonate per million. Upon adding copper sulphate to water treated in this manner very little basic copper carbonate is formed.

"The other factor which lowers the percentage of copper sulphate in solution is the addition of hydrant water which it seems necessary to add from time to time during the day to take the place of that removed by the lemons. The addition of this water not only lowers the percentage of copper sulphate in solution, but also adds more alkali, thus

causing more basic copper carbonate to be formed, which still further weakens the solution.

"In the study of this question it seemed necessary to find some method by which the water that is added during the day can be made to contain the proper amount of copper sulphate. At present, the best way that can be suggested to accomplish this end is the installation of a second tank, from which the water needed during the day can be drawn. This tank should be filled each morning with a solution of copper sulphate of the same strength as that used in washing the lemons and the solution should be made in the same manner."

Other Suggestions.—(*a*) Keep the humidity of the tents as low as possible without injury to the fruit. (*b*) Systematic inspection of stored fruit, especially from those groves that in the past have shown cottony rot. (*c*) Do not use packing boxes that have in the past been infected with cottony rot unless these are thoroughly disinfected or the stained, infected portions are replaced with new wood. From some preliminary work it has been shown that five per cent solution of formalin is the best disinfectant to use in sterilizing boxes. The following experiment shows the use of different germicides in the treatment of portions of boxes that became infected with cottony rot. The pieces measured about three inches long by one inch wide. There were five pieces tested in each case. The results given were after the treated pieces had been in moist chamber two weeks.

Germicide	Time	Results
Mercuric chloride 1/10%	10 seconds	No growth.
Mercuric chloride 1/10%	1 minute	No growth.
Mercuric chloride 1/10%	5 minutes	No growth.
Formalin 5%	10 seconds	No growth.
Formalin 5%	1 minute	No growth.
Formalin 5%	5 minutes	No growth.
Check	Untreated	Cottony rot.
Boiling water	10 seconds	Growth of penicillium and no evidence of cottony rot.
Boiling water	1 minute	
Boiling water	5 minutes	
Copper sulphate 5%	10 seconds	Growth of penicillium and evidence of cottony rot.
Copper sulphate 5%	5 minutes*	
Copper sulphate 5%	5 minutes	

* Copper sulphate was rinsed off at end of treatment five minutes.

(*d*) Contact fruit should be kept separate from other fruit where frequent attention can be given it. Some houses have found it desirable to employ specially trained men to handle virulent decays like cottony rot and brown rot.

GENERAL CONCLUSIONS

Research upon this disease has established the following facts:

1. Repeated inoculations have proved that the cottony rot is caused by a species of Sclerotinia, probably *Sclerotinia libertiana.*

2. That this fungus not only attacks the lemon fruit but also the twigs of mature and small orchard trees, nursery stock, and sweet and sour orange seed bed stock.

3. The white cottony filaments (mycelium) or the vegetative part of the fungus, are able to enter and destroy a perfectly sound lemon at any point of contact, no abrasion being necessary.

4. Several strains of *Sclerotinia libertiana* from different localities of the United States, when inoculated in the fruit and twigs of lemons are pathogenic, showing infection similar in all characteristics to that of lemon cottony rot isolated from citrus fruits and twigs.

5. Attempts to inoculate the healthy uninjured skin of a lemon with spores applied with an atomizer over the entire fruit, have failed except at the stem and blossom ends, and rarely at points where two fruits are in contact.

6. The fungus has repeatedly been carried through its entire life history from ascospore to ascospore. No other spore stage has been observed.

7. Strains of Sclerotinia isolated from bean, cucumber, lettuce, vetch, wild lettuce (*Latuca scariola*), citrus twigs, avocado twigs, tomato, and eggplant, appear to be identical with that causing the decay of lemon fruits known as the cottony rot.

8. No more satisfactory control has been discovered than disinfecting the fruits by the use of a wash water containing 1/50 per cent solution of copper sulphate, a method in general use for the control of brown rot.

9. It has been found important to neutralize the wash water by use of sulphuric acid before the copper sulphate is added, this being necessary to prevent the breaking down of the copper sulphate and the weakening of the disinfecting solution.

10. In replenishing the solution in the wash tank, more of the disinfecting solution properly made and of the required strength should be added.

11. Other important factors in controlling the injury from this disease in packing houses are: (1) the sterilization of storage boxes, (2) frequent inspection, especially of stored fruit from infested groves, (3) the isolation of fruit that may have been infected by contact.